Automation of Single Cell Plating Utilizing the Tempest

FORMULATRIX

App Note

Introduction

Cell line generation is generally considered a long and tedious process requiring a substantial investment of labor and materials. Monoclonality and robust clonal growth with high viability are desirable in cell line development, especially in the production of monoclonal antibodies and protein therapeutics. Automated cell dispensing and cytometry technologies enable increased throughput while reducing the time and labor required to dispense, identify and verify the presence of a single cell per well. This study compares the dispense and analysis of CHO cells to a 384-well plate using the automated Formulatrix Tempest liquid dispenser and the Brooks Celígo® S imaging cytometer versus electric hand pipetting and manual analysis.

Methods and Materials

I. Cell Culture

In order to prepare cells for single cell plating and clonal outgrowth, CHO-K1 YFP cells were adherently cultured in T-25 cm^2 flasks with F-12K media containing 10% FBS and 1% G418. Cells were incubated in 5% CO2 at 37°C and 98% relative humidity. To perform viability studies, suspension CHO (CHOs) cells were cultured in 125 mL Erlenmeyer flasks (Corning #430421) containing 30 mL CD CHO media (Gibco #10743) supplemented with 20mM GlutaMax (Gibco #35050-061) and 1% HT (Gibco #11067). Cells were incubated in 8% CO2 at 37°C and 98% relative humidity on an orbital shaker table (130 rpm).

II. Single Cell Plating with Tempest or Manual Pipet

CHO-K1 cells expressing the yellow fluorescent protein (YFP) were harvested with Trypsin-EDTA (0.25%/0.5 mM) and resuspended in culture media. Cells were passaged twice through a 40 µm cell strainer mesh (BD Falcon #352340) to achieve a cell suspension without aggregates, then counted using a hemocytometer and diluted to 200 cells/mL. Media and cells were plated using either the Tempest from Formulatrix or the hand-held Matrix electronic multi-channel pipette (Thermo Fisher #2231, 12-Channel 384 Equalizer Pipette, 2-125 µl).

The Formulatrix Tempest is a non-contact, bulk reagent dispenser configurable to simultaneously deliver any volume of up to 12 separate ingredients through 96 individually controlled nozzles. The Tempest's patent-pending microfluidic valve cluster dispenses discrete volumes of liquid using positive displacement technology and is capable of accurate and precise dispenses to 96-well, 384-well and 1536-well plates. In addition, using a standard pipette tip as the source reservoir reduces the dead volume to 100 µL, optimal for precious sample dispenses.

Prior to cell plating, 25 µL/well of culture media was dispensed into 384-well plates (Corning #3542). 5 µL/well of cell suspension was subsequently plated (n=3 for each dispensing method). Plates were centrifuged to collect cells at the bottom of each well, and incubated until imaged.

III. Imaging Single Cell Plating with Brooks Celígo S

Plates were processed for detection of single cell per well and clonal outgrowth using the Celígo imaging cytometer. The Brooks Celígo S imaging cytometer is a high-speed, easy-to-use, multichannel brightfield and fluorescence imaging cytometer for high-throughput, whole-well cellular image acquisition and

Formulatrix Tempest

processing of multi-well plates. The Celígo provides brightfield imaging and three channels of fluorescence imaging capabilities for visualizing and quantifying cellular responses in flasks and 6-well to 1536-well microplates. The system provides full resolution at 1 µm/pixel.

Developed for brightfield and fluorescence imaging, the entire Celígo incorporates proprietary optics and software to image and identify cells with consistent contrast across the whole well all the way to the well's edge. This enables identification of the position and area of the cells without requiring additional potentially cytotoxic fluorescent probes.

Plates were imaged at day 0 with 2-channel acquisition application using a fluorescent image for cell detection and a brightfield image for visual verification of single cell presence. After a four day incubation period, plates were imaged again in 2-channel acquisition application. Brightfield imaging was used to confirm clonal outgrowth.

IV. Viability and Plating Efficiency Counts on Celígo

Viability measurements were compared between the two dispensing methods after overnight recovery in an incubator. For viability determination after an overnight recovery, CHO cells were prepared into a cell suspension and pipetted into a 384-well plate (Corning 3542) at a concentration of 500 cells per well using the Tempest or the electronic pipette. Plates were incubated overnight. Concentrations of 5µg/mL (8uM) Hoechst 33342 (Life Technologies, #H3570) and 2µg/mL (3uM) of Propidium Iodide (PI) (Life Technologies, #P3566) were added to wells and incubated for 30 minutes. Plates were imaged and analyzed for viability with the Celígo.

Data and Results

I. Single Cell Plating

In order to compare the proper dispensing of cells between a hand-held electronic pipet and the Formulatrix Tempest, the number of wells where a single cell was detected was quantified. Manual plating with the electric pipet rendered 36% (3% CV) of wells with single cells, and the Tempest rendered 32% (2% CV) (figure 1). Both plating methods performed equally in single cell plating with no statistical differences ($P < 0.01$, $n=3$).

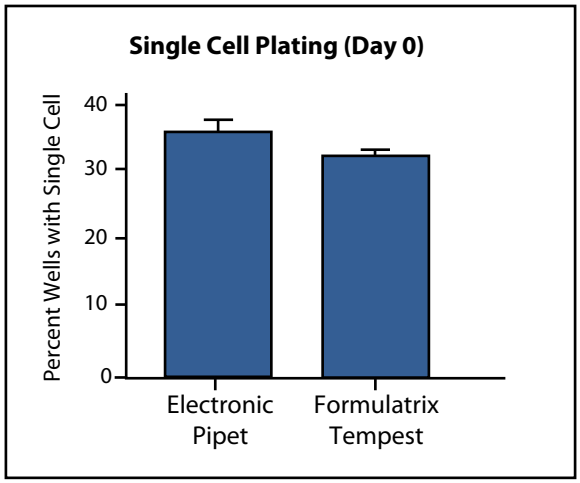

Figure 1. Comparison of single cell plating efficiencies between electronic pipet and the Tempest. CHO-K1 YFP cells were plated in 384-well plates with an electronic pipet or Formulatrix Tempest. After plating, the cell number was quantified in each well using the Celígo imaging cytometer and wells with single cells were reported for the Day 0 time point. Single cell per well plating occurred successfully 36% of the time (3% CV) with the electric pipette and 32% (2% CV) of the time with the Tempest.

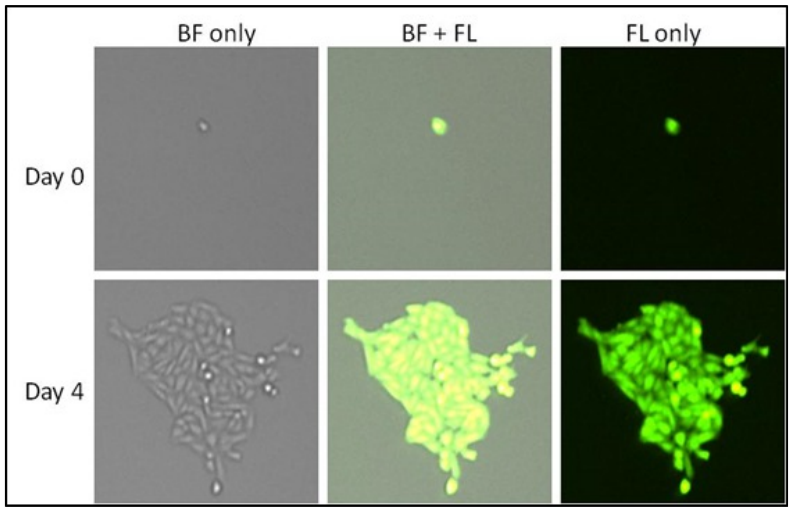

Figure 2. Brightfield (BF), fluorescent (FL) and merged images of clonal outgrowth. Celígo images of a single CHOK1 YFP cell (Day 0) that has grown into a colony (Day 4) in BF only (left), Merged BF+FL (middle), and FL only (right). Visual confirmation of clonality is easily monitored with the Celígo.

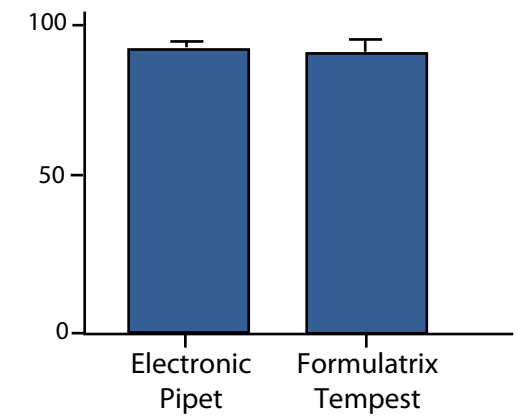

Figure 3. Graph of percent clonality between the electric hand-held pipet and the Formulatrix Tempest. The 384 wells with a single CHOK1 YFP cell were tracked using a Celígo imager for clonal outgrowth, comparing two methods of plating: the hand-held electric pipet and the Formulatrix Tempest. Both methods had close to 91% clonal outgrowth with no statistical differences (P< 0.01, n=3).

	Formulatrix Tempest	Electronic Pipet
Viability	97.4% (1.1% CV)	98.9% (0.7% CV)
Plating Variation	10% CV	17% CV

Table 1. Cell viability and plating variation with the Formulatrix Tempest in comparison with an electronic pipet. Viability measurements were taken from Celígo images of CHO-S cells stained with Propidium Iodide and Hoechst. Viability was assessed after overnight recovery. Reproducible plating of cells per well was examined in the viability plates. Plating CVs were 10% for Formulatrix Tempest and 17% for electronic pipetting. Plating cells with the Formulatrix Tempest or with the electronic pipet had comparable cell viability, 97.4% (1.1% CV) and 98.9% (0.7% CV).

II. Clonal Outgrowth

Comparison of clonal outgrowth was easily monitored with the Celígo by visually tracking at Day 0 and at Day 4 scans for wells with a single cell growing into a colony. Figure 2 shows representative images of a single cell growing into a colony. Both cells and colonies could be visualized in the brightfield and fluorescent channels of the Celígo. This allowed quantifiable evaluation of clonal outgrowth between the hand-held electronic pipet and the Formulatrix Tempest, for which 91% (6% CV) and 91% (4% CV) of single cells grew into a colony respectively (Figure 3). These results showcase that there is no statistical differences (P< 0.01, n=3) in clonal outgrowth between the two methods of dispensing cells.

III. Viability and Plating Efficiency on Celígo

For both plating methods, cell viability was monitored using CHO-S cells. With an overnight recovery period, viability measurements for the Tempest plating method was 97.4% (1.1% CV) and for the electronic pipet plating method was 98.9% (0.7% CV) (Table 1). Both methods recovered equally well after plating. In comparison of cell counts across the plate, the Formulatrix Tempest had better reproducibility with a 10% CV, whereas the electric manual pipet had 17% CV (Table 1).

IV. Manual vs. Automated Liquid Dispensing and Well Analysis

Automating the dispensing and well analysis process enables a substantial increase in throughput compared to manual dispensing and well analysis. Manually dispensing cells to a 384-well plate takes about 3 minutes and is subject to greater variability and human errors. The Tempest reliably dispenses cells to a 384-well plate in about 40 seconds, saving time and increasing accuracy.

The labor-intensive process of manually analyzing wells to verify the presence of a single cell requires approximately 30 seconds per well or 192 minutes for a 384-well plate, while the Celígo S completes the image acquisition and analysis process in about 7 minutes. Automated dispensing and analyzing a 384-well plate requires about 8 minutes whereas manual dispensing and analysis requires about 195 minutes. Accordingly, the automated process increases throughput about 25-fold to dispense cells and analyze the wells for the presence of a single cell (Figure 4).

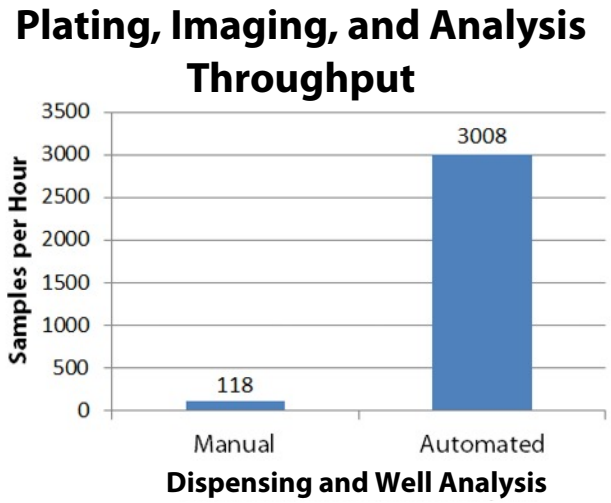

Figure 4. Throughput analysis of manual and automated dispensing and well analysis. Manual dispensing and well analysis enables an approximate throughput of 118 samples per hour while implementing an automated dispensing and well analysis systems increases the throughput approximately 25-fold to over 3000 samples per hour.

Conclusion

The purpose of this study was to assess the use of an automated liquid dispensing system with a plate-based cytometer for monoclonal cell line development. To develop monoclonal cell lines, one must first dispense the experiment plate and then verify that a single cell per well is present. These steps can be time consuming and laborious and can substantially increase experiment expenses.

Combining the Formulatrix Tempest liquid dispenser with the Brooks Celígo S imaging cytometer created a fast, efficient and reliable system for dispensing and identifying wells with single cells. The Formulatrix Tempest automatically dispensed cells to 384-well plates in fewer than 40 seconds per plate, which was more than two minutes faster than the electronic pipet plate dispense. In addition, use of the Tempest eliminated extra consumable costs for materials such as troughs and pipette tips required for manual dispenses.

Quantification of the number of cells or colony per well was performed by automated scoring with the Celígo S. Results analysis showed that the Formulatrix Tempest dispensed a single cell per well to 32% of the wells while maintaining cell viability. Using the two machines together produced a combined time savings of over 95% in comparison to manual pipet dispense and single cell per well analysis.